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(Article begins on next page)



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Boletus mendax, a new species of Boletus sect. Luridi from Italy and insights on the B. luridus complex

Alfredo Vizzini, Giampaolo Simonini, Enrico Ercole and Samuele Voyron

Abstract

The new species *Boletus mendax* of sect. *Luridi* is reported and discussed; its morphological, anatomical and edaphic characters are described and a molecular investigation is carried out in order to elucidate the separation from neighboring taxa. The present research also demonstrates the high degree of segregation of two collections of the recently described *Boletus comptus* and the low taxonomic value of the usual reddish pigmentation of the subhymenophoral layer (the so called Bataille's line) for both taxa *B. mendax* and *B. luridus*. Colour pictures taken in habitat of the taxa presented herein are also provided. Finally, according to an ITS analysis, sect. *Luridi* is shown to be polyphyletic and the importance of morphological features as stipe ornamentation, context colour beneath tubes and amyloid reaction in stipe base is discussed.

Introduction

Section *Luridi* Fr. ex Lannoy & Estadès, typified by *B. luridus* Schaeff., is a traditionally well-defined group in the genus *Boletus* L. (Boletaceae Chevall.), distributed mainly in temperate Europe (Singer 1967; Pilát and Dermek 1974; Engel et al. 1983; Alessio 1985; Lannoy and Estadès 2001; Muñoz 2005; Kłofac 2007; Šutara et al. 2009; Knudsen and Taylor 2012) and North/Central America (Coker and Beers 1943; Singer 1947; Snell and Dick 1970; Smith and Thiers 1971; Thiers 1975; Grund and Harrison 1976; Both 1993; Bessette et al. 1997, 2000; Ortiz-Santana et al. 2007), as well as temperate eastern and tropical southeastern Asia (Corner 1972; Zang 2006; Takahashi et al. 2011) and Australia (Watling and Li 1999). The species of this section are characterized by a boletoid habit, small, often orange-reddish pores, a reticulate or minutely furfuraceous stipe surface and usually blueing tubes, pores and context. According to a recent preliminary molecular analysis on Mediterranean *Boletus* (Marques et al. 2010), sect. *Luridi* is polyphyletic and split into three clades.

Within this section, *Boletus luridus* is distinguished in having a pileus variable in colour, olivaceous to snuff-brown, peach or brick-coloured to reddish brown, pores orange-red even when young, context red to blood-red at the base of tubes (due to the Bataille's line), stipe cylindric-clavate usually not robust and markedly swollen, coarsely and strongly reticulate with a red network overall and an amyloid reaction on basal stipe context (Singer 1967; Leclair and Essette 1969; Watling 1970; Pilát and Dermek 1974; Engel et al. 1983; Alessio 1985; Lannoy and Estadès 2001; Muñoz 2005; Watling and Hills 2005; Galli 2007; Kłofac 2007; Šutara et al. 2009; Kibby 2011; Knudsen and Taylor 2012).

The variability of *B. luridus* is puzzling: based only on morphological data it is hard to define whether, as currently delimited, it is a single, very variable taxon, or a collective species (Simonini 2001). In the latter case, what are the characters useful for their differentiation and at what taxonomic rank should we place the neighbouring taxa?

As it is well known, *B. luridus* displays a reliable chromatic character: the so-called Bataille's line (Lannoy and Estades 2001; Muñoz 2005), probably observed by Bataille (1908) for the first time, i.e., a dull red coloured thin layer between tubes and pileus context. Such a feature is not present in any other European and extra European species belonging to section *Luridi*, even those considered most similar and taxonomically allied to *B. luridus*. The real origin of the Bataille's line is to date, unknown, and the constancy of this character is somewhat questionable. Moreover, the opposite (and not necessarily antithetical) statement is also questionable: is it possible that more taxa can have the Bataille's line?

Our work aims to consider a new taxon having the Bataille's line (at least in the majority of cases) like *B. luridus*, but different from this latter species in the general appearance, colours, size of the spores, pileipellis structure and habitat. In addition, the monophyly of section *Luridi* and its morphologically based splitting in subsections and stirps (Lannoy and Estades 2001; Klofac 2007) are tested against the molecular phylogenetic approach.

Materials and methods

Macro- and microscopic features

Fresh basidiomata were photographed in the field to show their distinctive characters before being collected; notes on accompanying vegetation and on soil type and characteristics were taken. A Pentax K5 reflex digital camera with lens Pentax SMC D FA Macro WR 1:1 100 mm was used. Photographs were taken in day light or with flash Pentax AF 360 FGZ light. The colour of spore print was estimated the day after the deposit, in dry conditions. All the colour descriptions were based on Kornerup and Wanscher (1978).

Microscopic features were observed on well-dried material; free-hand sections of dried material were revived in L4 solution, Melzer's reagent (for spores) or in ammoniacal Congo red (pileipellis, hymenium). Measurements were made at 1000× magnification, with an ocular micrometer. Spore dimensions were taken from the pileus hymenophore of mature specimens, randomly chosen, and given as: (minimum–) average minus standard deviation – average plus standard deviation (–maximum) of length × (minimum–) average minus standard deviation – average plus standard deviation (–maximum) of width [μm]; $Q = (\text{minimum–}) \text{ average minus standard deviation – average plus standard deviation (–maximum) of ratio length/width}$, $Q_m = \text{average} \pm \text{standard deviation of ratio length/width}$, $V = (\text{minimum–}) \text{ average minus standard deviation – average plus standard deviation (–maximum) of the volume } [\mu\text{m}^3]$, $V_m = \text{average} \pm \text{standard deviation of the volume } [\mu\text{m}^3]$. The approximate, conventional spore volume was estimated as a rotation ellipsoid with the formula: $V = 4/3 * (\text{length}/2) * ((\text{width}/2) * \text{width}) * \pi/2$. To assess spore size, at least 31 spores per collection were measured. The notation (c = X) means that measurements were made on X collections; standard deviation, maximum and minimum are expressed with reference to the X average values. After measurement of dimensions, spores were further tested for amyloidity in Melzer's reagent. Anatomical microscopical features were observed on glass slides with a Jenamed Variant optical light microscope, and all line-drawings of microstructures were made from rehydrated material. Photographs are taken from the microscope through a Jena phototube extension. Amyloid reaction of the context with Melzer's reagent was tested in the stipe base following Imler's procedure (Imler 1950; Singer 1965).

Specimens of the type collection examined in this study were deposited in AMB (isotype in GS). Herbarium acronyms follow Thiers (2013) except "GS" that refers to the personal herbarium of

Giampaolo Simonini. Author citations follow the Index Fungorum-Authors of Fungal Names (<http://www.indexfungorum.org/authorsoffungalnames.htm>).

The infra-generic (sectional) taxonomic classification of *Boletus* follows Muñoz (2005).

DNA extraction, PCR amplification, and DNA sequencing

Genomic DNA was isolated from 1 mg of 10 herbarium specimens (in succession from AMB12632 to AMB12641) using the DNeasy Plant Mini Kit (Qiagen, Milan Italy). Universal primers ITS1F/ITS4 were used for the ITS region amplification (White et al. 1990; Gardes and Bruns 1993). Amplification reactions were performed in PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems) in a 25 µl reaction mixture using the following final concentrations or total amounts: 5 ng DNA, 1 × PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 1 µM of each primer, 2.5 mM MgCl₂, 0.25 mM of each dNTP, 0.5 unit of Taq polymerase (Promega). The PCR program was as follows: 3 min at 95 °C for 1 cycle; 30 s at 94 °C, 45 s at 50 °C, 2 min at 72 °C for 35 cycles, 10 min at 72 °C for 1 cycle. PCR products were resolved on a 1.0 % agarose gel and visualized by staining with ethidium bromide. PCR products were purified and sequenced by MACROGEN Inc. (Seoul, Republic of Korea). Sequence assembly and editing were performed using Geneious v5.3 (Drummond et al. 2010). Sequences were deposited in GenBank under the accession numbers given in Table 1.

Table 1

Boletus sequences newly generated for this study and associated GenBank accession numbers

Species	Collection data Voucher	Herbarium n.	ITS GenBank acc. n.
B. mendax 1	24.08.10, Agriturismo Rio Riccò, Busana (RE), <i>Castanea sativa</i> , leg. G. Simonini	AMB12632	KC734547
B. mendax 2	27.08.10, Rifugio Zamboni, Villa Minozzo (RE), <i>Fagus sylvatica</i> , leg. G. Simonini	AMB12633 (Holotype)	KC734548
B. mendax 3	24.08.10, Il Castagno, Busana (RE), <i>Castanea sativa</i> , leg. G. Simonini	AMB12634	KC734543
B. mendax 4	28.08.10, Agriturismo Rio Riccò, Busana (RE), <i>Castanea sativa</i> , leg. G. Simonini	AMB12635	KC734545
B. mendax 5	10.10.11, unkn. loc. in Appennino reggiano, leg. unkn.	AMB12637	KC734540
B. mendax 6	09.11.2011, Sardinia, Calangianus (OT), <i>Quercus suber</i> , leg. G. Simonini	AMB12640	KC734541
B. luridus 1	28.08.10, Rio Riccò, Busana (RE), <i>Castanea sativa</i> , leg. G. Simonini	AMB12636	KC734542
B. luridus 2	21.08.10, M. Torricella, Villa Minozzo (RE), <i>Quercus pubescens</i> , leg. unkn.	AMB12638	KC734544
B. comptus	18.08.10, Calizzo, Villa Minozzo (RE), <i>Quercus pubescens</i> , leg. G. Simonini	AMB12639	KC734539
B. queletii	26.09.10, Intermesoli, Pietra Camela (TE), <i>Quercus</i> sp., <i>Ostrya carpinifolia</i> , <i>Castanea sativa</i> , leg. G. Simonini	AMB12641	KC734546

Sequence alignment and phylogenetic analysis

Sequences included in the phylogenetic analyses were either generated in this study or retrieved from GenBank (<http://www.ncbi.nlm.nih.gov/>) and UNITE (<http://unite.ut.ee/index.php?e0true>) databases. *Boletus aereus* (UDB000943), *B. aestivalis* (UDB001113), *B. edulis* (UDB011153, UDB000944) and *B. pinophilus* (UDB000939) were used as outgroup taxa. Alignments were generated using MAFFT (Kato et al. 2002) with default conditions for gap openings and gap extension penalties. The sequence alignments were slightly refined manually with MEGA 5.0 (Tamura et al. 2011). Phylogenetic analyses were performed using the Bayesian Inference (BI) and Maximum Likelihood (ML) approaches. The BI was performed with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001) with four incrementally heated simultaneous Monte Carlo Markov Chains (MCMC) run over 10 million generations, under GTR + Γ evolutionary model. Trees were sampled every 1,000 generations resulting in an overall sampling of 10,001 trees; the first 2,500 trees were discarded as “burn-in” (25 %). For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian posterior probabilities (BPP).

ML estimation was performed through RAxML v.7.3.2 (Stamatakis 2006) with 1,000 bootstrap replicates (Felsenstein 1985) using the GTRGAMMA algorithm to perform a tree inference and search for a good topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the “-f a” option of RAxML and “-x 12345” as a random seed to invoke the novel rapid bootstrapping algorithm.

BI and ML analyses were run on the CIPRES Science Gateway web server (Miller et al. 2010). Only BPP values over 0.70 and MLB over 50 are reported in the resulting tree (Fig. 1). Branch lengths were estimated as mean values over the sampled trees. Pairwise % identity values (P%IV) of ITS sequences were calculated using MEGA 5.0 (Tamura et al. 2011).

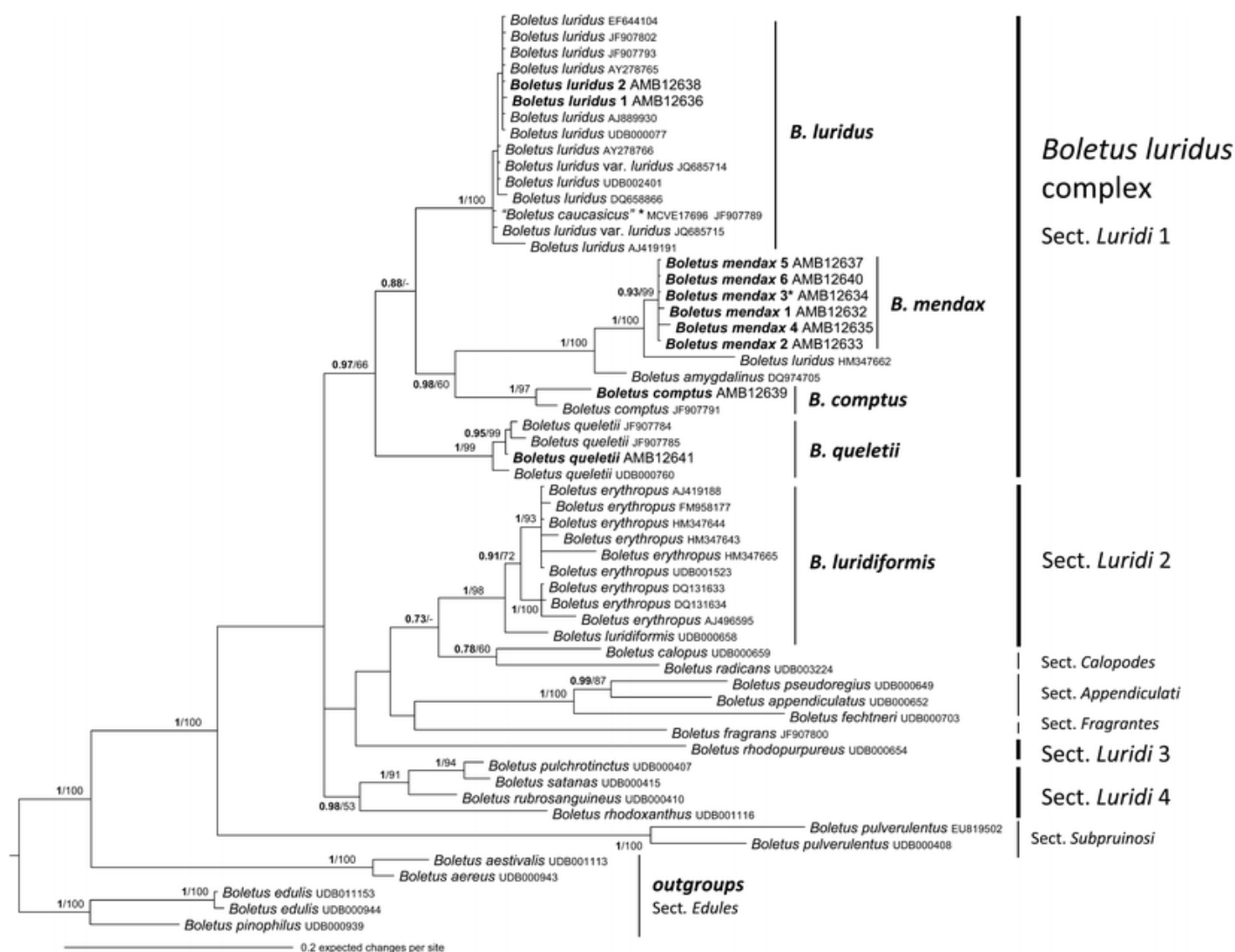


Fig. 1

Bayesian phylogram obtained from the general nrITS sequence alignment of *Boletus* spp. Support values in either the Bayesian (Posterior probabilities values [BPP]) or Maximum likelihood (ML bootstrap percentage [MLB]) analyses are indicated. Only BPP values over 0.70 (in bold) and MLB values over 50 are given above clade branches. Newly sequenced collections are in bold. Numbers refer to the collections cited in Tab. 1. * indicates collections of *B. luridus* and *B. mendax* without Bataille's line

Results

Phylogenetic analysis

Both Bayesian and Maximum likelihood analyses produced the same topology; therefore, only the Bayesian tree with both BPP and MLB values is shown (Fig. 1). The ITS dataset comprised a total of 57 sequences (including 26 from GenBank and 21 from UNITE). The alignment comprised 978 characters, and contains 522 variable sites.

In the obtained Bayesian phylogram (Fig. 1), sequences from collections belonging to sect. *Luridi* do not form a monophyletic assemblage and are split into four clades (Sect. *Luridi* 1-4). The six sequences of *B. mendax* form, together with "*Boletus luridus* HM347662" from Portugal and *B. amygdalinus* from North America, a well-supported and distinct clade (BPP = 1.0, MLB = 100); this clade is sister to the *B. comptus* clade (BPP = 1.0, MLB = 97), and both (BPP = 0.98, MLB = 60) are sister to the *B. luridus* clade. The *B. queletii* clade (BPP = 1.0, MLB = 99) is sister to all the previously cited clades. *B. luridus*, *B. mendax*, *B. amygdalinus*, *B. comptus* and *B. queletii* form a

well-supported assemblage (BPP = 0.97, MLB = 66), hereafter refereed as the *B. luridus* complex (sect. *Luridi* 1).

The P%IV is 99.6 for the 6 sequences of *B. mendax*, 98.9 for the 15 sequences of *B. luridus*, 99.0 for the 2 sequences of *B. comptus*, and 98.4 for the 4 sequences of *B. queletii*. The *Boletus luridus* HM347662 sequence vs. the sequences of *B. mendax* shows a P%IV of 95.4. *B. amygdalinus* DQ974705 vs. the *Boletus luridus* HM347662 sequence and *B. mendax* sequences shows a P%IV of 94.4, while of 95.4 vs. only *B. mendax* sequences.

From the obtained BI (Fig. 1) and ML trees, the 10 sequences of *B. luridiformis* (*B. luridiformis* clade, sect. *Luridi* 2), form at least three distinct clades probably ascribable to three different taxa.

Taxonomy

Boletus mendax Simonini & Vizzini, sp. nov. (Figs. 2a-e, 3, 4, 5 g-i, 6 and 7)

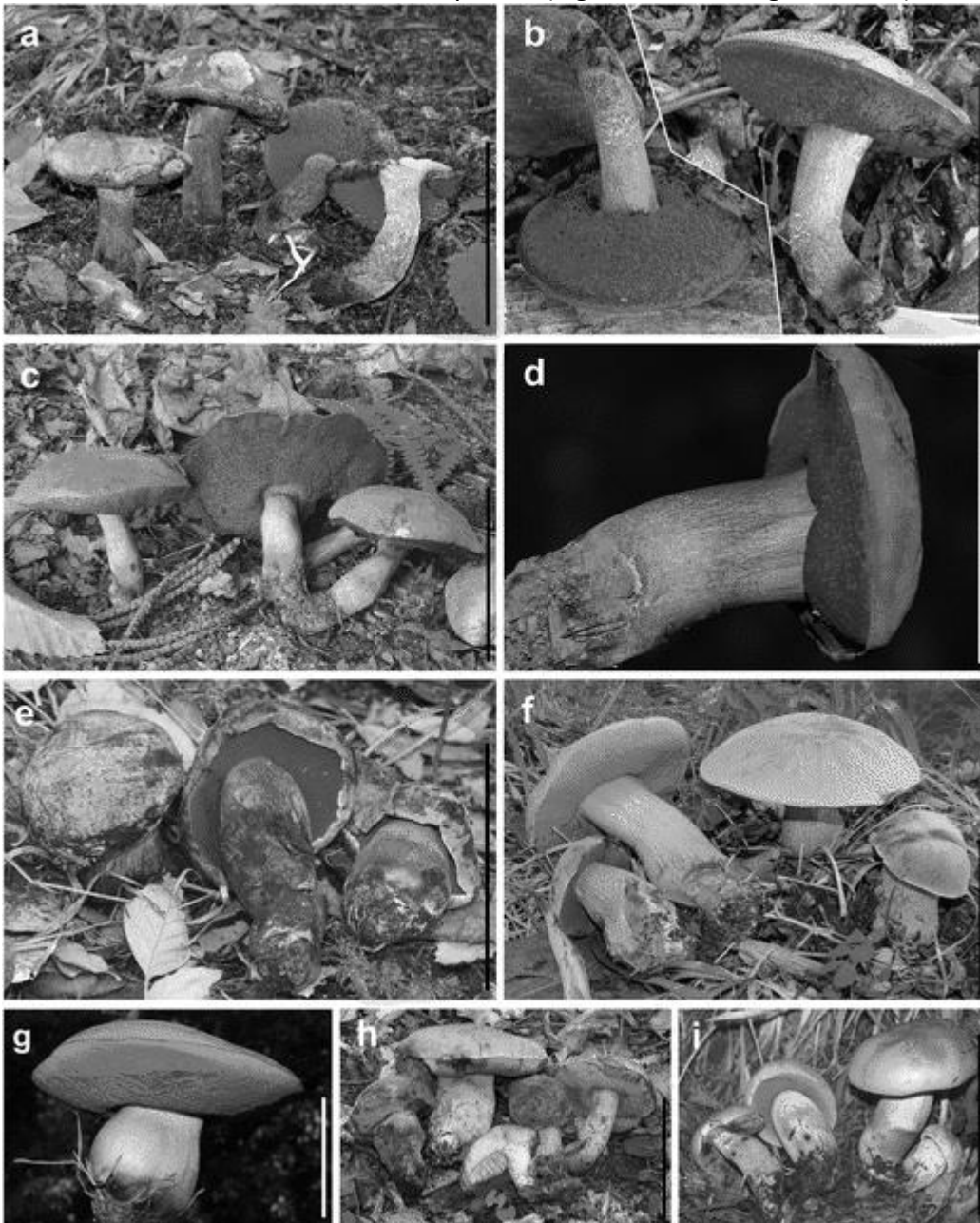


Fig. 2

Basidiomata of the ITS sequenced collections. a *Boletus mendax* (AMB 12633, holotype). b *Boletus mendax* (AMB 12635). c *Boletus mendax* (AMB 12632). d *Boletus mendax* (AMB 12634). e *Boletus mendax* (AMB 12640). f *Boletus luridus* (AMB 12636). g *Boletus luridus* (AMB 12638). h *Boletus queletii* (AMB 12641). i *Boletus comptus* (AMB 12639). Bars = 10 cm

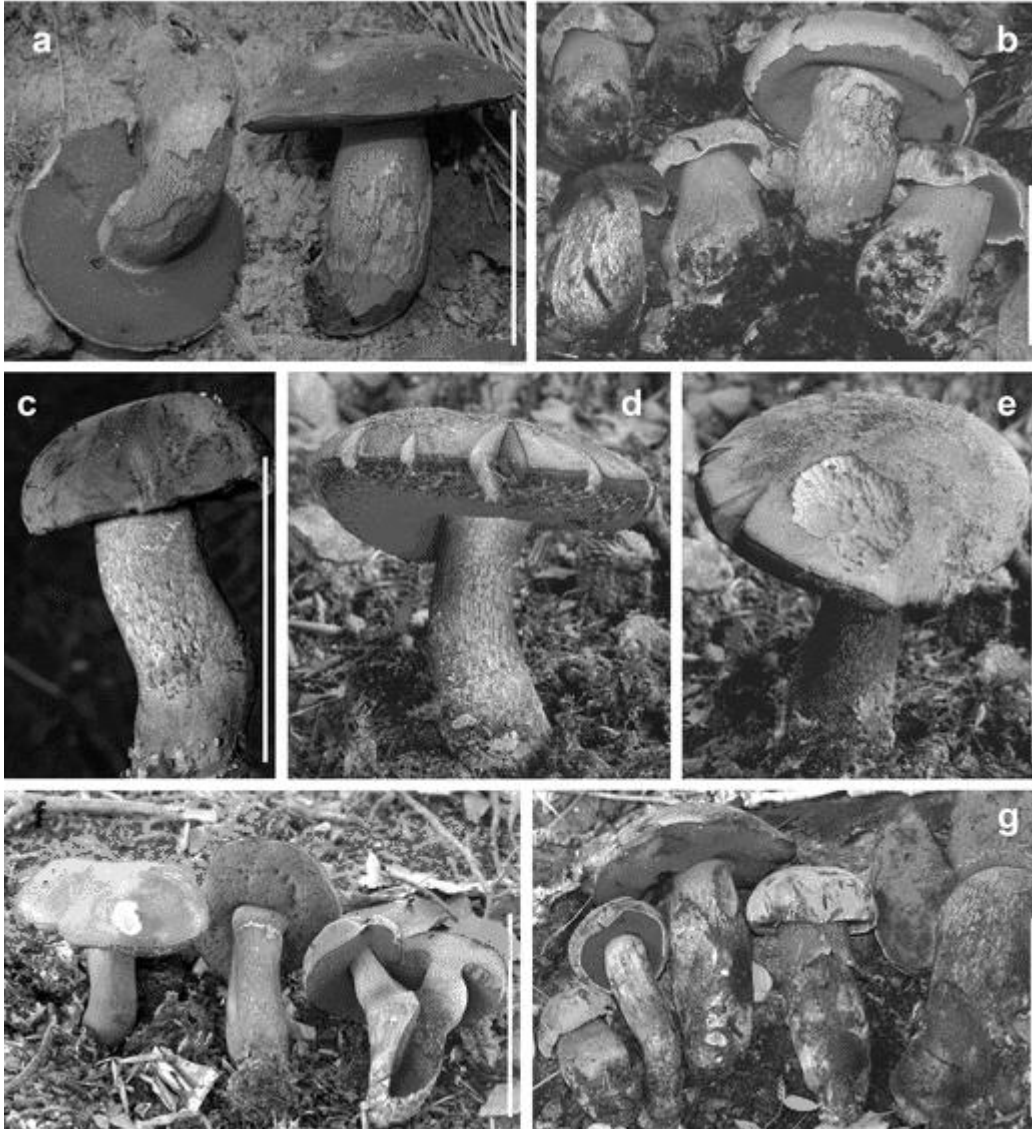


Fig. 3

Other collections of *B. mendax*. a Coll. GS10004, atypical, with a well defined reticulum on the stipe, with *Castanea sativa*. b Coll. GS0915, tough basidiomata from *Quercus cerris*. c Coll. GS10039, habit resembling *B. luridiformis*, with *Castanea sativa*. d and e Coll. GS10012, typical small-sized basidiome of high altitudes, from Appennino Tosco Emiliano, on soil of “macigno” sandstone decay, with *Fagus sylvatica*. f Coll. GS10010, again typical small-size aspect from Appennino Tosco Emiliano, from the same collection site of the type collection. g Coll. GS10047, large specimens under *Quercus suber*, granitic soil, Sardinia, from the same site of coll. AMB12640. Bars = 10 cm

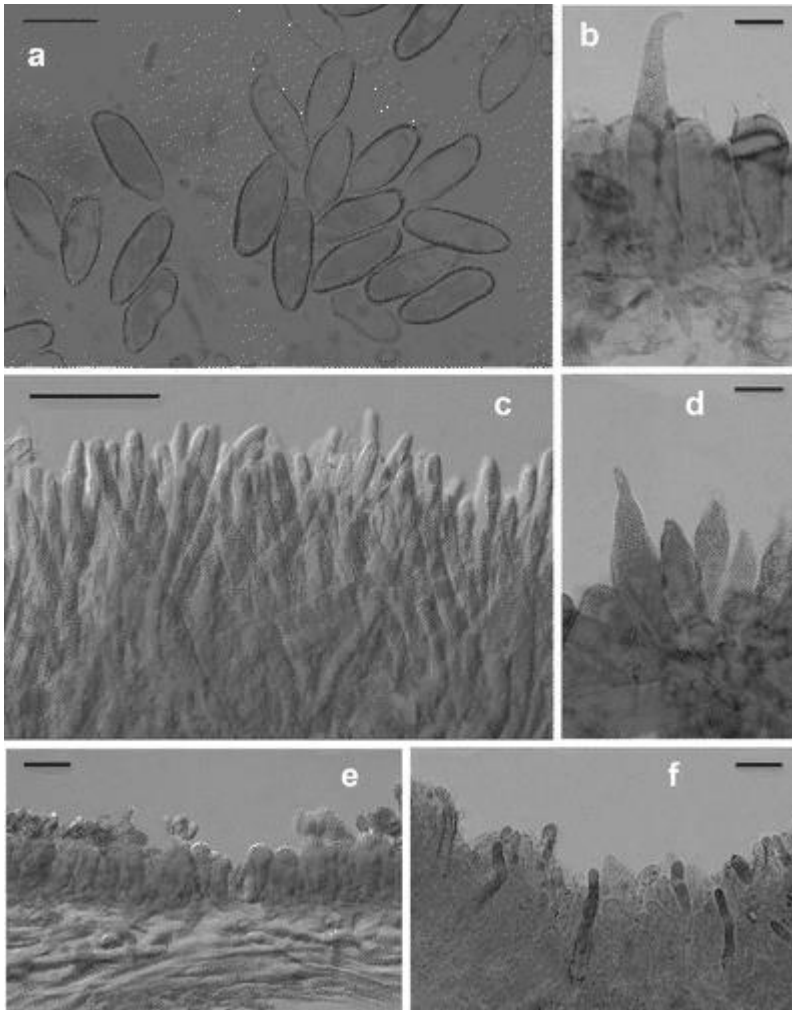


Fig. 4
Boletus mendax. Microscopic features (all from the type collection, AMB 12633). a Spores, in L4. b Facial cystidia, in Congo red. c Pileipellis, in Congo red with interferential phase contrast. d Marginal cystidia, in Congo red. e Basidia, in Congo red, with interferential phase contrast. f Pseudocystidia, in Congo red. Bars: a, b, d, e, f = 10 μ m; c = 50 μ m

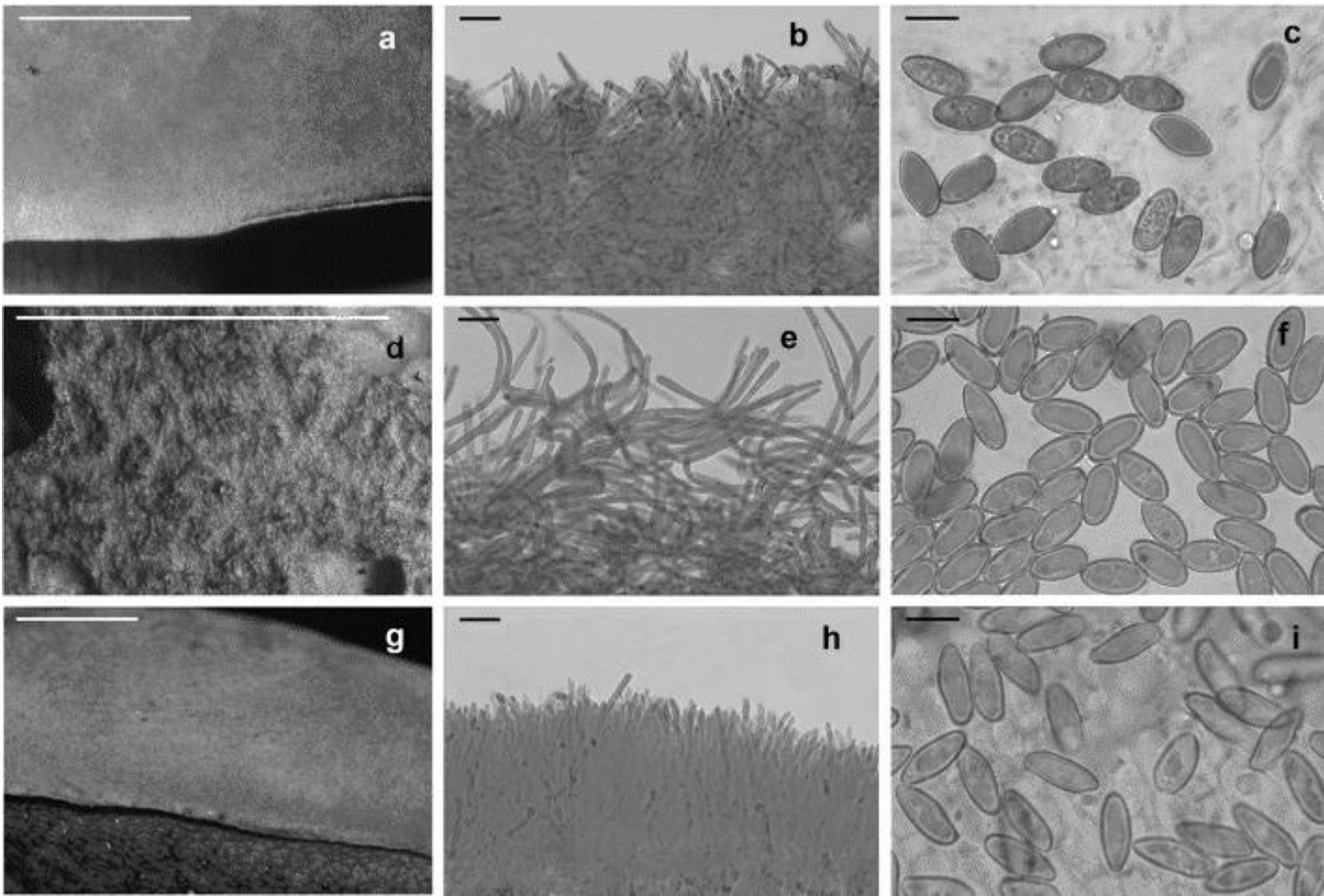


Fig. 5

Comparison among the main anatomical features of *Boletus luridus* (a-c, GS0875, AMB12636, MCVE17561), *B. comptus* (d-f, MCVE17872, GS10067, GS0978) and *B. mendax* (g-i, AMB12634, AMB12634, MCVE18095). a, d, g Magnification of the pileipellis structure. b, e, h Pileipellis structure, cross section at the mid radius of the pileus, in Congo Red. c, f, i Mature spores, in L4. Bars: a, d, g = 1 cm; b, e, h = 50 μm; c, f, i = 10 μm

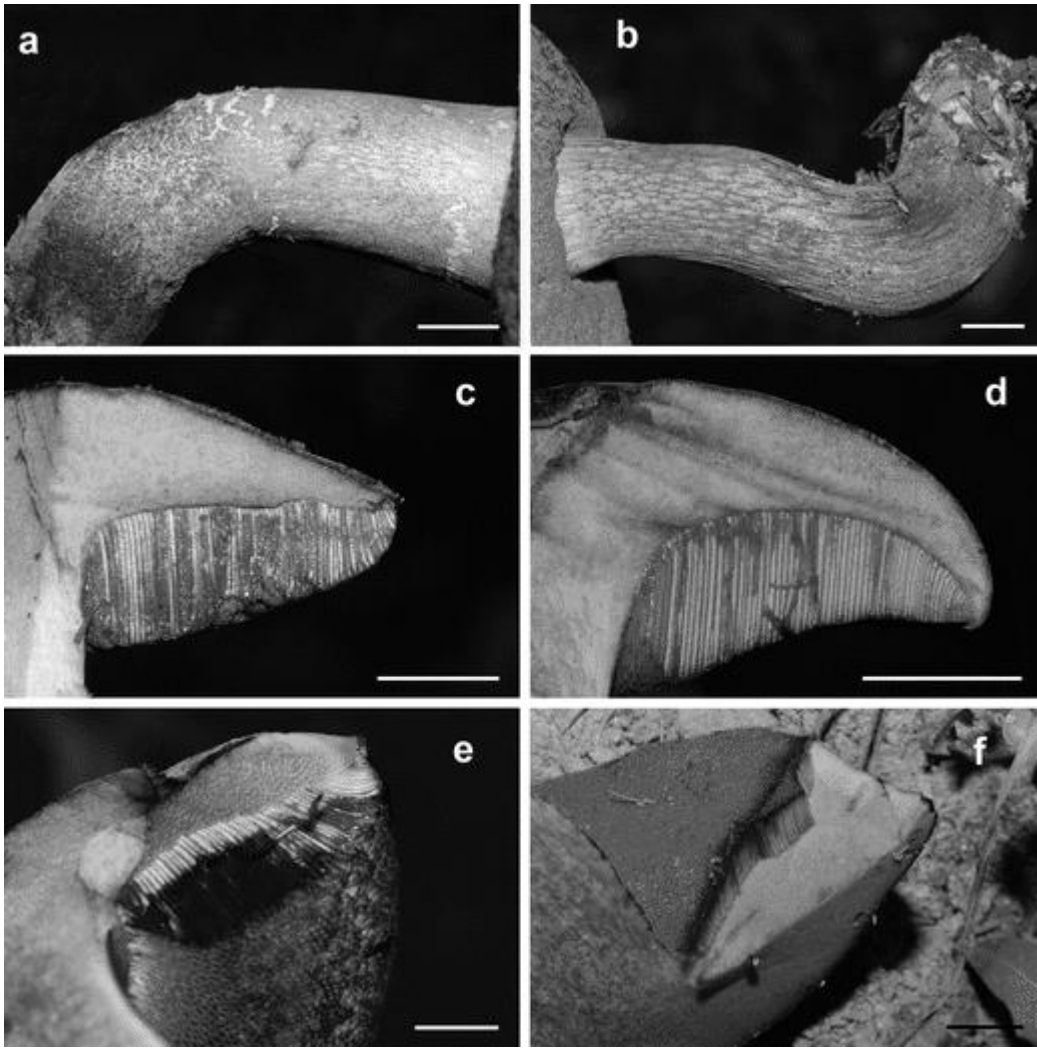


Fig. 6

Details of basidiomata of *B. mendax*. a Coll. AMB12635, stipe with a very faint reticulum in the upper part and scurfy granules in the lower one. b Coll. AMB 12633 (holotype), stipe with a more defined reticulum and granules restricted at the extreme bottom. c Coll. GS10011, context with evident Bataille's line. d Coll. AMB12634, context without Bataille's line. e Coll. GS10012, the red layer below tubes. f Coll. GS10004, the lack of the red layer below tubes. Bars = 1 cm

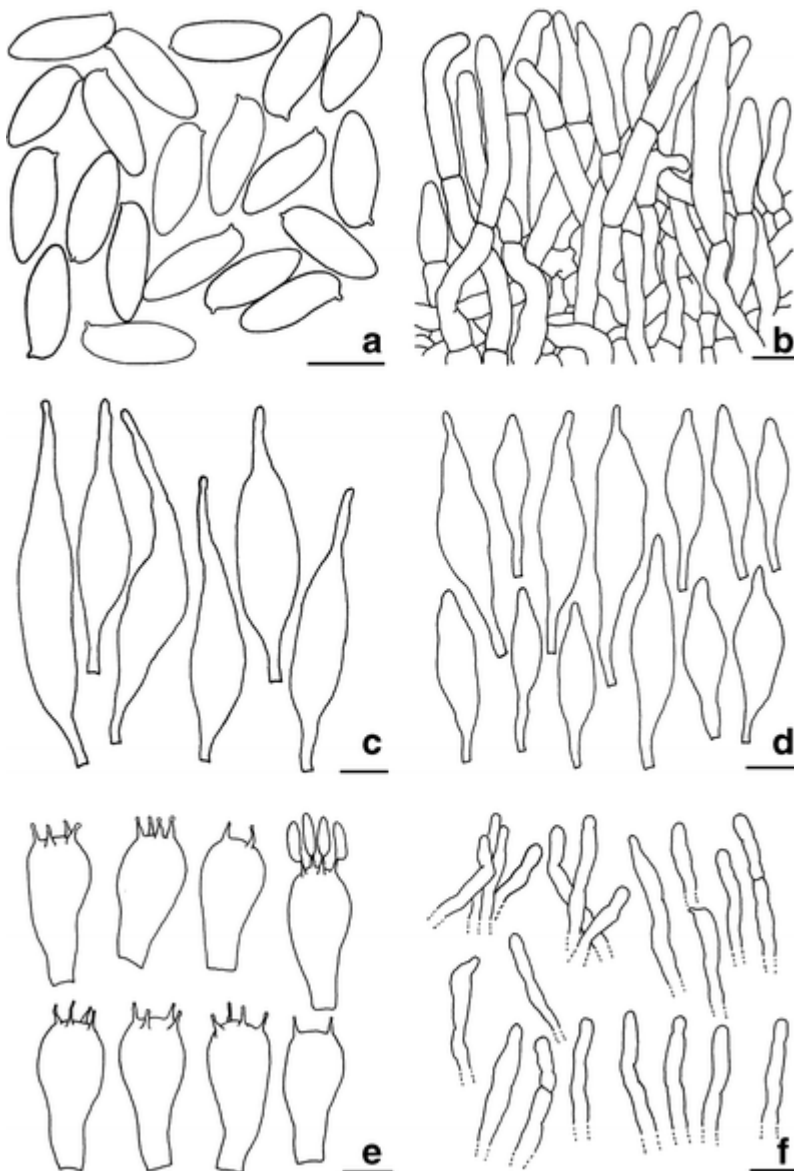


Fig. 7
Boletus mendax. Line drawings of microscopic features (all from the type collection, AMB 12633). a Spores. b Pileipellis end-elements. c Facial cystidia. d Marginal cystidia. e Basidia. f Pseudocystidia. Bars = 10 µm

Mycobank MB 803606

Etymology from Latin, *mendax* = mendacious, false, which refers to the resemblance of the species to *Boletus luridus*.

Original diagnosis:

The species is reminiscent of *Boletus luridus*, usually having a red, crimson-red stipe, rarely orange-yellow or yellow, with a reticulation frequently limited at the upper part which is often poorly defined and not evident. Below reticulation, scurfy granules similar to those of *B. luridiformis* occur. The pileus denotes intense and dominant reddish, crimson red-pink colours, often with shades of buff, brownish or olivaceous. The pileus surface is velvety or tomentose, never smooth or viscous. The context is tough, pale yellow, on cutting turning to dark indigo-blue, especially at the stipe base where the colour change is overlaying the natural beetroot colour, with a usually reddish subhymenophoral layer. Pores red to vivid scarlet red, with tones brighter than those of *B. luridus*, fading with age. The spores are quite different in shape from those of *B. luridus*, more elongated ($Q_m = 2.7$ with respect to $Q_m = 2.2$ of *B. luridus*) and tending to the *B.*

luridiformis spore shape; the terminal elements of pileipellis are typically straight and erected and only later, when fully mature, interwoven. Amyloid reaction at the stipe basis strongly positive. It grows in acidic soil, with *Fagus sylvatica*, *Castanea sativa* or, less frequently, *Quercus cerris*, *Q. suber* and *Q. ilex*.

Holotype (here selected): Italy, Emilia Romagna, Provincia of Reggio Emilia, Comune of Villa Minozzo, loc. "Rifugio Zamboni", with *Fagus sylvatica*, 1150 m a.s.l., 27 Aug. 2010, legit. G. Simonini (AMB 12633); isotypus GS10009.

Detailed description - Macrocharacters Pileus 40–120(–200) mm, hemispherical then convex, rarely plane, with an obtuse, often undulate-lobate margin, colour very variable depending on weather conditions. Young basidiomata usually show a pale buff-olive colour (ranging from 5B2 to 5D4) tending, in dry conditions, to darken to drab olive (6E4 to 6D8), otherwise in wet weather staining to crimson everywhere (10D4 to 10D6) or sometimes with the centre persisting pale buff-olive (typically 5C3, 5B2) and the margin pale crimson-pinkish-incarnate (7A5 to 7B3). When moist, young basidiomata initially show a crimson-pinkish-gray colour (8C5 to 9C2), then, due to dry weather, fade to hazel-buff colour (6D5 to 7C8) and finally darken to tobacco (7E6 to 7E8). In shady forests young basidiomata also show a reddish to brown-reddish colour (9D8 to 8D7) darkening in old specimens to tobacco or sometimes to colours resembling those of *B. luridiformis*, brown-bay (7F8 to 8F8) with reddish patches (8D7) or olive areas (5D5), usually with a paler margin (7D5). In general, pileus colours of *B. mendax* overlap in a wide range those of *B. luridus*, even if *B. mendax* is darker and more frequently shows crimson-reddish colours. Surface velvety, then tomentose, never smooth or viscous, quickly staining dark blue to blackish when bruised (in wet weather). Stipe 40–120 × 8–30(–50) mm, cylindric, sometimes flexuous or slightly subventricose, attenuate at the base, somewhat rooting; surface orange-red, yellow only on the very upper part, very rarely orange-yellow or yellow everywhere, soon entirely red, crimson red, purple-red, darker towards the base, with a reticulation which is often poorly defined and limited to the upper part, rarely covering the entire stipe, darker than the ground colour. The lower part of the stipe becomes finely scurfy granular, the granules being of a dark-scarlet or dark purple-red colour and sometimes also partially overlayed by the reticulum. At the very base the stipe can be covered in older specimens by a snuff-brown, velvety tomentum. Tubes 5–12(–18) mm, rounded, adnexed to nearly free, chrome yellow then olivaceous, dark blue on cutting. Pores small, roundish, concolorous with tubes in the early developmental stage, very soon dark red-brownish (ranging from 8E8 to 9F6), then vivid scarlet red (8D7 to 9C8), dark blue on pressure, discolouring with age to a pale orange-olive (5D8 to 6D6). Pores appear to be more brightly coloured than those of *B. luridus*. Context lemon to chrome yellow, more intense in the pileus and stipe connection, beetroot at the stipe base, immediately indigo blue when exposed, very intense and darker in the lower part of the stipe and especially at the base, then fading to drab white. In rare cases the context in the pileus and upper part of the stipe can show dull-purplish red colours. Subhymenophoral layer of the same colour of the pores but not infrequently yellow (more than 20 % of the collections), especially with dry weather or in larger specimens. The context is typically tougher, heavier and elastic than in *B. luridus*, reminding that of *B. luridiformis*. Taste and smell weak, pleasant but not distinctive. Spore-print tobacco brown with an olive shade (6E-F8).

Microcharacters Spores subfusiform, showing a weak but always present suprahilar depression in side view, ellipsoid in front view, (12.4–)13.3–14.7(–15.5) × (4.5–)4.9–5.5(–5.7) μm , $Q = (2.5–)2.6–2.8(–2.9)$, $Q_m = 2.68 \pm 0.11$, $V = (156–)173–231(–255) \mu\text{m}^3$, $V_m = 202 \pm 29 \mu\text{m}^3$ ($c = 23$), golden yellow in water or L4, brown with a lilac shade in Melzer's reagent. Basidia usually 4-spored, rarely 2-spored, hyaline, rarely yellowish, $24.9–30.5 \times 9.5–12.7 \mu\text{m}$ ($c = 23$). Facial cystidia hyaline to pale yellow, fusiform, $60.0–69.1 \times 11.1–12.9 \mu\text{m}$ ($c = 23$). Marginal cystidia similar, shorter, $40.2–62.4 \times 10.2–13.3 \mu\text{m}$ ($c = 23$). Pseudocystidia are also present, mostly worm shaped, having a dark

yellow amorphous content and often with no evident septa at the base, protruding into the lateral layer or even into the medium layer of the tube trama, 4.8–6.2 μm broad. Pileipellis consisting of erect, parallel, cylindrical hyphae with tapered end cells, 26.4–46.1 \times 5.5–7.9 μm ($c = 23$), forming a regular, then more intermingled palisade, often not collapsing even in aged specimens, with intracellular pale brown pigment and no relevant incrustations. Hymenophoral trama of the “*Boletus*-type” according to Singer’s (1967) definition. Clamp connections not observed. Amyloid reaction at the stipe base strongly positive, violet blue-black, with the same intensity of *B. luridus* and *B. queletii*.

Habitat gregarious, thermophilic, not rare under *Fagus sylvatica* and *Castanea sativa* usually in summer and early autumn (late autumn in collections from Sardinia) but observed also under *Quercus cerris*, *Q. suber* and *Q. ilex*, mainly in acidic soils. Collected in many sites in Emilia Romagna, Tosco-Emiliano Appennine, at altitudes ranging from 800 (500 m a.s.l. in collections from Sardinia) to 1,200 m a.s.l., often on siliceous soil (soil from “macigno” sandstone decay) but probably widespread, since it was observed in some mushroom exhibitions in northern Italy. To date never collected under pure conifers.

Known distribution So far known only from Italy: Sardinia, Abruzzo, Tuscany, Emilia Romagna, Trentino Alto Adige, Piedmont; probably widespread throughout Europe in temperate regions.

Examined material of *Boletus MENDAX*. ITALY: 16.08.92, Le Borelle, Villa Minozzo (RE), *Fagus sylvatica*, leg. G. Nuccio, GS0911; 12.09.92, Ponte Tavarone (MC), *Quercus cerris*, leg. G. Simonini, GS0915; 30.07.94, Rio Ri, Vidiciatico (BO), *Fagus sylvatica*, leg. G. Consiglio, MCVE18095; 08.08.94, M. Prampa (RE), *Fagus sylvatica*, leg. F. Franceschetti, MCVE17744; 08.08.94, Prà Fenio, Ligonchio (RE), *Fagus sylvatica*, leg. F. Franceschetti, MCVE17745; 27.07.95, Rio Ri, Vidiciatico (BO), *Fagus sylvatica*, leg. G. Consiglio, MCVE18097; 04.08.95, Rio Ri, Vidiciatico (BO), *Fagus sylvatica*, leg. G. Consiglio, MCVE17848; 08.08.95, La Magolese, Villa Minozzo (RE), *Fagus sylvatica*, leg. G. Nuccio, MCVE17849; 03.09.95, Lagoni (PR), *Fagus sylvatica*, leg. unkn., GS1427; 16.09.95, Ceva (CN), *Fagus sylvatica*, leg. unkn., GS1441; 31.10.99, Bottigli (GR), *Quercus ilex*, leg. M. Sarnari, MCVE17209; 23.08.10, Il Castagno, Busana (RE), *Castanea sativa*, leg. G. Simonini, GS10004; 24.08.10, Agriturismo Rio Riccò, Busana (RE), *Castanea sativa*, leg. G. Simonini, AMB12632; 24.08.10, Il Castagno, Busana (RE), *Castanea sativa*, leg. G. Simonini, AMB12634; 27.08.10, Rifugio Zamboni, Villa Minozzo (RE), *Fagus sylvatica*, leg. G. Simonini, AMB12633 (Holotype), GS10009 (Isotype); 27.08.10, Rifugio Zamboni, Villa Minozzo (RE), *Fagus sylvatica*, leg. G. Simonini, GS10010; 27.08.10, Rifugio Zamboni, Villa Minozzo (RE), *Fagus sylvatica*, leg. G. Simonini, GS10011; 27.08.10, Le Borelle, Villa Minozzo (RE), *Fagus sylvatica*, leg. G. Simonini, GS10012; 28.08.10, Agriturismo Rio Riccò, Busana (RE), *Castanea sativa*, leg. G. Simonini, AMB12635; 10.10.11, unkn. loc. in Appennino reggiano, leg. unkn., AMB12637; 09.11.11, Casa Cantoniera, Calangianus (OT), *Quercus suber*, leg. G. Simonini, AMB12640; Casa Cantoniera, Calangianus (OT), *Quercus suber*, leg. G. Simonini, GS10047; Casa Cantoniera, Calangianus (OT), *Quercus suber*, leg. G. Simonini, GS10048.

Additional examined material – *Boletus luridus*. ITALY: 04.09.83, Calizzo (RE), *Quercus pubescens*, leg. G. Simonini, MCVE17285; 21.07.91, Miscoso, Ramiseto (RE), *Fagus sylvatica*, leg. L. Mantovani, MCVE17561; 17.10.91, M. Amiata (SI), leg. L. Cocchi, GS858; 20.06.92, Bergonzano, Quattro Castella (RE), *Quercus pubescens*, leg. G. Simonini, MCVE17921; 21.06.92, loc. unkn., leg. unkn., GS0875; 21.06.92, loc. unkn., leg. unkn., GS0876; 21.06.92, loc. unkn., leg. unkn., GS0877; 25.06.92, loc. unkn., leg. unkn., GS0882; 12.07.92, loc. unkn., leg. unkn., GS0890; 11.08.92, Le Borelle, Passo Cisa, Villa Minozzo (RE), *Fagus sylvatica*, leg. G. Simonini, GS0895; 11.08.92, Le Borelle, Passo Cisa, Villa Minozzo (RE), *Fagus sylvatica*, leg. G. Simonini, GS0896; 11.08.92, Le Borelle, Villa Minozzo (RE), *Fagus sylvatica*, leg. G. Simonini, GS0897; 13.08.92, Presa Alta, Ligonchio (RE), *Fagus sylvatica*, leg. G. Simonini, GS0900; 12.09.93, Calizzo, Villa Minozzo (RE), *Quercus pubescens*, leg. G. Simonini, GS0984; 19.09.93, Parco di Roncolo, Quattro Castella (RE),

Quercus pubescens, leg. G. Simonini, GS0999; 10.09.95, Vezzano (RE), *Quercus pubescens*, GS1433; 21.07.91, Lagastrello, Ramiseto (RE), *Fagus sylvatica*, leg. G. Simonini, GS0814; 28.08.10, Rio Riccò, Busana (RE), *Castanea sativa*, AMB12636; 21.08.10, M. Torricella, Villa Minozzo (RE), *Quercus pubescens*, AMB12638; 02.11.12, Tenuta Le Costantine, Casamassella (LE), *Quercus ilex*, GS10155. *B. luridus* collections attributable to *B. erythroteron*. ITALY: 22.08.99, Castelvechio, Appiano (BZ), *Fagus sylvatica*, leg. G. Simonini, GS7652. *B. luridus* collections attributable to “ *B. caucasicus* ” s. auct. ITALY: 27.07.86, Calizzo (RE), *Quercus pubescens*, leg. G. Simonini, MCVE17250; 06.07.94, Vallisnera, Ramiseto, (RE), *Quercus cerris*, *Castanea sativa*, leg. G. Simonini, MCVE17696. SWITZERLAND: 07.07.92, Flach, Cant. Zurigo, *Abies normanniana*, *Picea abies*, *Fagus sylvatica*, leg. C. Lavorato, MCVE17968. *B. luridus* collections attributable to *B. luridus* var. *lupiniformis*: 12.07.92, loc. unkn., leg. unkn., GS0889; 13.11.94, Su Gologone, Oliena, (NU), *Quercus ilex*, leg. G. Redeuilh, MCVE17846. *B. luridus* collections attributable to *B. luridus* var. *queletiformis*: 06.08.94, Cereggiò (RE), *Quercus cerris*, *Fagus sylvatica*, *Castanea*, leg. G. Simonini, GS1221; 25.08.99, Castelvechio, Appiano (BZ), *Fagus sylvatica*, leg. G. Simonini, MCVE17205; 28.08.2010, Busana (RE), *Quercus cerris*, *Castanea sativa*, leg. G. Simonini, GS100014. *B. luridus* collections attributable to *B. queletii* var. *pseudoluridus*. ITALY: 14.07.93, Corniglio (PR), *Fagus sylvatica*, leg. ignoto, GS0952; 13.08.02, Le Borelle, Villa Minozzo (RE), *Fagus sylvatica*, leg. G. Nuccio, GS2456. FRANCE: 21.10.95, Apt, Aix en Provence, *Quercus pubescens*, leg. G. Manavella, MCVE17890. SWITZERLAND: 16.08.95, Col de la Croix, Ollon Villars, Cant. Vaud, *Picea abies*, leg. G. Nuccio, MCVE18886. *B. luridus* collections attributable to *B. luridus* var. *rubriceps*: 09.09.95, Cugnale di Falcone, Acri (CS), *Quercus cerris*, *Pinus calabrica*, leg. C. lavorato, MCVE17969; 25.08.99, Castelvechio, Appiano (BZ), *Castanea sativa*, leg. G. Simonini, GS2125. *B. luridus* f. *primulicolor*. ITALY: 09.11.94, Monte Pizzinnu (NU), *Quercus ilex*, leg. U. Bonazzi, MCVE17859 (typus), GS1307 (isotypus); 21.06.97, Parco di Roncolo, Quattro Castella (RE), *Quercus pubescens*, leg. G. Nuccio, MCVE18182. SPAIN: 22.10.87, Madremanya, Girona, *Quercus ilex*, leg. J.M. Vidál, MCVE18533; 23.10.87, Torroella de Montgri Olivar, Girona, *Quercus coccifera*, leg. J.M. Vidál, MCVE18418.

– *BOLETUS COMPTUS*. ITALY: 06.09.87, Parco di Roncolo, Quattro Castella (RE), *Quercus pubescens*, leg. G. Simonini, MCVE17357 (Holotype), GS0466 (Isotype); 06.09.87, Begonzano, Quattro Castella (RE), *Quercus pubescens*, leg. G. Simonini, MCVE17358; 08.09.87, Capedro Calizzo, Villa Minozzo (RE), *Quercus pubescens*, leg. G. Simonini, MCVE17360; 08.09.87, Capedro Calizzo, Villa Minozzo (RE), *Quercus pubescens*, leg. G. Simonini, GS0477; 13.09.87, Capedro Calizzo, Villa Minozzo (RE), *Quercus pubescens*, leg. G. Simonini, MCVE17598; 13.09.87, Capedro Calizzo, Villa Minozzo (RE), *Quercus pubescens*, leg. G. Simonini, MCVE17599; 28.10.90, Cala Gonone, Dorgali (NU), *Quercus ilex*, leg. G. Simonini, GS0776; 18.10.91, Castello del Guardasone, Traversetolo (PR), *Quercus pubescens*, leg. G. Simonini, GS0859; 25.10.92, Case di M. Duro, Casina (RE), *Quercus pubescens*, leg. G. Simonini, GS0918; 12.09.93, Capedro Calizzo, Villa Minozzo (RE), *Quercus pubescens*, leg. G. Simonini, GS 0976; 12.09.93, Calizzo, Villa Minozzo (RE), *Quercus pubescens*, leg. G. Simonini, GS0978; 12.09.93, Capedro Calizzo, Villa Minozzo (RE), *Quercus pubescens*, leg. G. Simonini, GS0979; 19.09.93, Parco di Roncolo, Quattro Castella (RE), *Quercus pubescens*, leg. G. Simonini, GS0991; 19.09.93, Parco di Roncolo, Quattro Castella (RE), *Quercus pubescens*, leg. G. Simonini, GS0993; 19.09.93, Parco di Roncolo, Quattro Castella (RE), *Quercus pubescens*, leg. G. Simonini, GS0996; 19.09.93, Parco di Roncolo, Quattro Castella (RE), *Quercus pubescens*, leg. G. Simonini, GS0997; 19.09.93, Parco di Roncolo, Quattro Castella (RE), *Quercus pubescens*, leg. G. Simonini, GS0998; 19.09.93, Parco di Roncolo, Quattro Castella (RE), *Quercus pubescens*, leg. G. Simonini, GS1002; 19.09.93, Parco di Roncolo, Quattro Castella (RE), *Quercus pubescens*, leg. G. Simonini, GS1004; 13.11.94, Su Gologone, Oliena (NU), *Quercus ilex*, leg. G. Redeuilh, GS1350; 03.09.95, Pantano, Carpineti (RE), *Quercus pubescens*, leg. G. Sassi,

MCVE17870; 10.09.95, Case di M. Duro, Casina (RE), *Quercus pubescens*, leg. G. Simonini, MCVE17871; 10.09.95, Case di M. Duro, Casina (RE), *Quercus pubescens*, leg. G. Simonini, MCVE17872; 02.10.98, Calizzo, Villa Minozzo (RE), *Quercus pubescens*, leg. G. Simonini, GS1957; 19.09.99, Calizzo, Villa Minozzo (RE), *Quercus pubescens*, leg. G. Simonini, GS2145; 18.08.10, Calizzo, Villa Minozzo (RE), *Quercus pubescens*, leg. G. Simonini, AMB12639; 23.09.2012, Calizzo, Villa Minozzo (RE), *Quercus pubescens*, leg. G. Simonini, GS10067. *B. comptus* collections attributable to *B. luridus* var. *lupiniformis*. ITALY: 13.11.94, Cala Gonone, Dorgali, (NU), *Quercus ilex*, leg. G. Redeuilh, GS1350. SPAIN: 22.10.87, Madremanya, Girona, *Quercus ilex*, leg. J.M. Vidál, MCVE18531; 22.10.87, Madremanya, Girona, *Quercus ilex*, leg. J.M. Vidál, MCVE18530; 23.10.87, Torroella de Montgri, Girona, *Quercus coccifera*, leg. J. M. Vidál, MCVE18449; 15.10.94, Sant Felin de Pallais, Girona, *Quercus pubescens*, leg. J.M. Vidál et M.A. Perez-de-Gregorio, MCVE18532.

– *Boletus luridiformis* : collection attributable to “ *B. caucasicus* ” s. Singer. ITALY: 16.09.95, Ceva (CN), *Fagus sylvatica*, *Picea abies*, leg. unknown, GS1444; 16.10.98, Abetina Reale, Civago (RE), *Abies alba*, *Fagus sylvatica*, leg. G. Visentin, GS2026.

– *Boletus queletii*. ITALY: 12.09.1987, Cerreto, Viano (RE), *Quercus pubescens*, *Q. cerris*, leg. G. Simonini, GS0485; 12.09.1987, Pulpiano, Viano (RE), *Quercus cerris*, leg. G. Simonini, GS0486; 20.09.1987, Calizzo, Villa Minozzo (RE), *Quercus pubescens*, leg. G. Simonini, MCVE17655; 30.07.90, Monte Maccione, Oliena (NU), *Quercus ilex*, leg. G. Simonini, MCVE17656; 13.06.1992, Pulpiano, Viano, RE, *Quercus cerris*, leg. G. Simonini, GS0870; 16.08.1992, Le Borelle, Villa Minozzo (RE), *Fagus sylvatica*, leg. G. Simonini, GS0907; 30.08.1992, Marola, Carpineti (RE), *Castanea sativa*, leg. G. Simonini, GS0912; 20.09.1993, loc. unkn., leg. P. Barigazzi, GS1003; 16.07.1994, Pulpiano, Viano (RE), *Quercus cerris*, leg. G. Simonini, MCVE17719; 24.09.1994, Trinità, Casina (RE), *Tilia cordata*, leg. M. Comuzzi, MCVE17762; 19.09.1999, Calizzo, Villa Minozzo (RE), *Quercus pubescens*, leg. G. Simonini, MCVE17208; 06.08.2000, Cecciola, Ramiseto (RE), *Castanea sativa*, leg. G. Simonini, MCVE18552; 26.09.2010, Intermesoli, Pietra Camela (TE), *Quercus* sp., *Castanea sativa*, AMB12641.

Discussion

The status of *Boletus* sect. *Luridi*

Based on morphological features, most authors used for the species with orange-red pores a unique section *Luridi* without further subdivisions in subsections or stirps (Singer 1967; Pilát and Dermek 1974; Engel et al. 1983; Alessio 1985; Singer 1986; Muñoz 2005; Šutara et al. 2009; Knudsen and Taylor 2012). Bertéa and Estadès (1990), Cazzoli (1991) and Redeuilh (1992) informally delimited the species strongly bruising blue-black overall on handling as group *Torosus*, stirp *Torosus* and stirp *Inquinans*, respectively; more recently, Hlaváček (1996) formally and validly delimited this latter group as subsect. *Torosi* of the sect. *Luridi* (cfr. Simonini and Bertolini 2012). Andary et al. (1992), in a chemotaxonomic study, informally recognized the subsection *Satanas* (nom. inv.) for the large species containing diastereomers of the 2-amino-4-hydroxypentanoic acid; Lannoy and Estadès (2001), after having stressed the importance of characters such as the stipe ornamentation types, the reaction of the context to Melzer's reagent, the presence of a red coloured layer above the tubes and bruising reaction types as key characters for delimiting subsectional taxa, divided this section into five informal “*Séries*”, *Luridus*, *Permagnificus*, *Punctipedes*, *Satanas* and *Torosus*; this placement was also followed by Klofac (2007). Finally, sect. *Luridi* was considered heterogeneous by Watling and Hills (2005) and split into sect. *Satani* and *Purpurei* of subg. *Suillellus* and sect. *Luridi* and *Luridiformes* of the subg. *Luridellus*; Galli (2007)

established sect. *Erythropodes* for the species without a reticulate stipe. As traditionally delimited (Singer 1967; Pilát and Dermek 1974; Engel et al. 1983; Alessio 1985; Singer 1986; Lannoy and Estades 2001; Muñoz 2005; Kľofac 2007; Šutara et al. 2009; Knudsen and Taylor 2012), sect. *Luridi* is clearly a polyphyletic assemblage, as already shown by Binder and Hibbett (2006, Supplementary Fig.1) and Marques et al. (2010), and the species of this section are distributed over at least four clades (Fig. 1). Section *Luridi* 1 (*B. luridus* complex) includes *B. luridus* Schaeff., *B. mendax*, *Boletus luridus* HM347662, *B. amygdalinus* (Thiers) Thiers, *B. comptus* Simonini and *B. queletii* Schulzer, taxa characterized by usually slender basidiomata, cylindric stipe with or without reticulum (*B. amygdalinus* and *B. queletii*), usually ochre-brown to olivaceous, reddish pileus bruising blue-black when handled, presence or absence of Bataille's line, context red in the stipe base and strongly blueing when exposed to air and strong amyloid reaction (no data for *B. amygdalinus*, Thiers 1975; Bessette et al. 2000) (Lannoy and Estades 2001; Muñoz 2005; Kľofac 2007; Knudsen and Taylor 2012); section *Luridi* 2 (corresponding to sect. *Erythropodes* Galli partim, Galli 2007) consisting only of *B. luridiformis* Rostk. (= *B. erythropus* sensu Auct.) sequences (grouped in three distinct subclades), an easily recognizable species with a club-shaped stipe covered with dense orange-red floccules, non amyloid context and spores with a $Q_m = 2.65\text{--}2.68$ (Lannoy and Estades 2001; pers. obs.); section *Luridi* 3 consisting of *B. rhodopurpureus* Smotl., representative of a complex of species bruising deep blue-black overall and none to weak amyloid reaction (Lannoy and Estades 2001; Muñoz 2005), corresponding to group *Torosus* sensu Bertéa and Estadès (1990), stirp *Torosus* sensu Cazzoli (1991), stirp *Inquinans* sensu Redeuilh (1992), subsect. *Torosi* Hlaváček (1996), Série *Torosus* sensu Lannoy and Estades (2001) and sect. *Purpurei* sensu Watling and Hills (2005); section *Luridi* 4 encompasses *B. pulchrotinctus* Alessio, *B. satanas* Lenz, *B. rubrosanguineus* Cheype and *B. rhodoxanthus* (Krombh.) Kallenb., species with large basidiomes, pileus white to greyish or grey-buff to milky coffee, not blueing (reddening at most), context only weakly blueing, very swollen stipe and none to weak amyloid reaction (Lannoy and Estades 2001; Muñoz 2005), corresponding to subsect. *Satanas* sensu Andary et al. (1992), Série *Satanas* sensu Lannoy and Estades (2001) and sect. *Satani* sensu Watling and Hills (2005).

In the light of our ITS data, all these authors overstressed the importance of some characters such as the stipe ornamentation (presence/absence of reticulum) and/or presence of Bataille's line for delimiting subsections or stirps. For example, série *Punctipedes* of sect. *Luridi* (Lannoy and Estades 2001), sect. *Luridiformes* (Watling and Hills 2005) and sect. *Erythropodes* (Galli 2007) were established to accommodate species with a nonreticulate stipe; séries *Luridus* and *Permagnificus* (Lannoy and Estades 2001) for reticulate amyloid taxa with or without Bataille's line, respectively. But phylogenetic analyses (Fig. 1) showed that species with different stipe ornamentation (e.g. *B. luridus*/*B. queletii*) or different coloration of the context beneath tubes (e.g. *B. mendax*/*B. comptus*) may belong to the same clade and conversely taxa with similar stipe and colours (e.g. *B. luridiformis*/*B. queletii*) may be attributable to different phylogenetic groups. On the contrary, the presence of an amyloid reaction in the stipe base seems to be a reliable taxonomically informative character.

***Boletus mendax* and allied species**

According to morphological data and phylogenetic analyses of ITS sequences, *Boletus mendax* should be regarded as an independent species within *Boletus* s.l. The new species is clearly distinguished from the other taxa by having a unique combination of morphological and edaphic characters including: a velvety pileus with prevailing reddish, crimson red-pink colours (Fig. 3), a usually reddish subhymenophoral layer (Figs. 6c, e), vivid scarlet-red pores (Fig. 3), a reticulum often poorly defined and often limited to the upper part (Fig. 6a), pale yellow context turning dark

indigo blue on cut (Figs. 3b, f), stipe base red (Fig. 2a) and strongly amyloid, elongated spores ($Q_m = 2.7$) (Figs. 4a, 5i, 7a), pileipellis consisting of a regular trichodermium with the end-elements typically straight and erect (Figs. 4c, 5h, 7b), and the growth in acidic soils.

ITS sequence analyses (Fig. 1) suggest a close affinity of our new species with the sequence labeled *Boletus luridus* HM347662 and *B. amygdalinus*. The first sequence, obtained by Marques et al. (2010) from a Portuguese collection, shows a P%IV of 95.4 and could be considered a new undescribed species in the complex; future morphological analyses could provide evidence for considering this collection as distinct from *B. mendax* at the specific level. The North American *B. amygdalinus* differs from *B. mendax* by a stipe without reticulum, a subhymenophoral layer always without Bataille's line, larger spores ($11.2\text{--}16 \times 5.2\text{--}8 \mu\text{m}$) and a pileipellis differentiated as a confuse trichodermium of interwoven hyphae (Thiers 1965, as *B. puniceus*; Thiers 1975; Bessette et al. 2000).

The species that are morphologically similar to *B. mendax* are *B. luridus*, *B. comptus*, "*B. caucasicus*" and *B. queletii*, briefly discussed below.

Boletus luridus

Schaeff. (Schaeffer 1774) is a well known and quite common species, widespread in neutral-calcareous soils and described by the synthetic original diagnosis that gives the following descriptive characters (translated from German): "Dirty yellow pileus. Tubes yellow to greenish, with red mouth. Stipe cylindric, stout, yellowish in the upper part, often red in the lower, with a brown reticulum, dark at the base, rooting". Starting from the original description of the colour of the pileus ("dirty yellow pileus"), different authors described this taxon (Persoon 1825; Gilbert 1931; Blum 1968) gradually enlarging its chromatic range up to olivaceous, olivaceous-brown, buff, with reddish shades, almost never uniform but with the above mentioned colours intermixed and producing many halftones.

The pileus surface is finely velvety, but smooth in old specimens and also slightly viscous in humid weather. The reticulum covering the stipe is sharp, with wide and longitudinally stretched meshes, often composite, on a yellow or orange-yellowish ground. The pileus context, just above the hymenophoral layer, shows a typical red, orange red or pinkish colour, usually of the same tint of the pores, originating the characteristic "Bataille's line" when observed in cross section.

Some differences with respect to *B. mendax* can be found in pileus colour, in the latter usually being brown-reddish to crimson-red, even if the colour range of *B. luridus* and *B. mendax* can at times overlap. More evident are the differences in stipe surface decorations and colours: it is mainly red (yellow or orange-yellow tints occur in the apical part of young specimens) in *B. mendax*, while it is mainly yellow or orange-yellow (even if tending to red in medium-lower part) in *B. luridus* (however, exceptions must be taken into account in both cases). Extension and relief of the reticulum of *B. mendax* is very variable: some collections show a well outlined red network upon a red ground, covering the surface up to the stipe base (Fig. 6b); however, in many cases the reticulum is restricted to the upper part of the stipe, and is weakly defined (Fig. 6a); the reticulum of *B. mendax* shows usually simple, not compounded meshes. Moreover, the reticulum in the stipe of *B. mendax* is frequently replaced by a fine dark red scurfy punctuation laying upon a red coloured ground and resembling those of *B. luridiformis*. Such fine granules are partially superimposed on the reticulum and distributed up to the lower part of the stipe where they are replaced by a dirty-brownish velvety tomentum (Figs. 6a, b).

The occurrence of Bataille's line is very erratic in *B. mendax*. Most of the specimens show an evident Bataille's line when cut (Fig. 6c) and, in correspondence, the layer below the tubes appears red coloured (Fig. 6e). However, more than 20 % of our collections do not show any Bataille's line, even if the lack of this character could at least be partly due to dry weather. Collections of *B. mendax* without such feature are usually named "*B. caucasicus*" Singer (Figs. 3a

and 6f, coll. GS10004; 2d and 6d, coll. AMB 12634). However, we had the evidence of a really fresh collection (GS10047) recorded in wet conditions (Fig. 3f) in which Bataille's line was present in abt. 50 % of the specimens, regardless to the stage of development. In *B. luridus* the Bataille's line can also be absent, even if this occurrence is very rare. Molecular phylogenetic analyses have clearly shown that in both *B. mendax* and *B. luridus* the lack of Bataille's line has no taxonomic value (see below, under "*Boletus caucasicus*", and Fig. 1, collections *B. mendax* AMB 12634 and "*B. caucasicus*" MCVE17696).

The spores of *B. luridus* are ellipsoid, having a length/width ratio much smaller ($Q = 2.1\text{--}2.4$; $Q_m = 2.2$; $c = 24$) than that of *B. mendax* ($Q = 2.6\text{--}2.8$; $Q_m = 2.7$; $c = 23$), (Table 2, Fig. 5c, i). In *B. luridus* the pileipellis consists of an interwoven trichodermium becoming more and more confused with age and eventually tending to collapse (Fig. 5a-b). Conversely, *B. mendax* shows an erect, parallel trichodermium (palisade), tending only in aged specimens to be intermingled (Fig. 5g-h). Pileipellis end-cells diameter in *B. mendax* ($5.5\text{--}7.9\ \mu\text{m}$) is close to that of *B. luridus* ($5.0\text{--}7.5\ \mu\text{m}$) and *B. comptus* ($4.8\text{--}7.1\ \mu\text{m}$) (the latter two indicative values are taken from Simonini 1993).

Table 2
Spore size comparison between *B. comptus*, *B. luridus* and *B. mendax*

Species	Average length [μm]	Average width [μm]	Q (length/width) [-]	Qm (average length/width) [-]	Vm [μm^3]
<i>Boletus comptus</i> (c = 29)	12.6 ± 0.7	5.9 ± 0.3	(1.8-)2.0-2.3(-2.6)	2.16 ± 0.12	231 ± 30
<i>Boletus luridus</i> (c = 28)	12.8 ± 0.7	5.8 ± 0.3	(1.9-)2.1-2.4(-2.5)	2.22 ± 0.14	227 ± 31
<i>Boletus mendax</i> (c = 23)	14.0 ± 0.7	5.2 ± 0.3	(2.5-)2.6-2.8(-2.9)	2.68 ± 0.11	202 ± 29

All known European subspecific taxa as yet described and attributed to *B. luridus* variability (i.e. *Boletus erythroteron* ("erythroteron", "erythrenteron") Bezděk (1901) [= *Boletus luridus* var. *erythroteron* (Bezděk) Pilát & Dermek (Dermek 1979); = *Boletus luridus* subsp. *erythroteron* (Bezděk) Hlaváček (1995)], *Boletus luridus* var. *lupiniformis* J. Blum, *Boletus luridus* var. *queletiformis* J. Blum (Blum 1968), *Boletus luridus* var. *rubriceps* (Maire) Dermek (1987) (= *Tubiporus luridus* var. *rubriceps* Maire 1937), *Boletus luridus* f. *primulicolor* Simonini (Simonini and Lavorato 1997) show either the pileus colour or the extension and relief of the reticulum as the only distinctive characters from *B. luridus* (see "Additional examined material, *B. luridus*"); in our opinion, none of them can be considered a realistic previous synonym for *B. mendax*.

Boletus comptus

Simonini (Simonini 1993) is another close taxon to the new species. Even if pinkish tones could sometimes be present on *B. comptus* pileus, this bolete is quite different from *B. mendax*. In particular, pores are red-orange, orange, yellowish-orange or even yellowish, much paler than the bright red colour of *B. mendax* pores. In both *B. mendax* and *B. comptus* the stipe is poorly reticulate and scurfy-granulose below the net (even not reticulate at all in *B. comptus*), but in the latter species the stipe is orange-yellow to yellowish, never red or crimson-red as frequently occur in *B. mendax*. The stipe base is strongly tapered and rooting in *B. comptus*, only attenuate in *B. mendax*. In *B. comptus* Bataille's line is generally absent, even if a reddish-pink layer under the tubes might occasionally be observed (Simonini 1993). The spores of *B. comptus* ($Q = 2.0\text{--}2.3$; $Q_m = 2.16$; $c = 29$) show practically the same shape and size of *B. luridus*, whilst *B. mendax*

($Q = 2.6\text{--}2.8$; $Q_m = 2.7$; $c = 23$) produces spores significantly longer, thinner and more slender (Table 2, Fig. 5f, i). The pileipellis structure is also quite different: in *B. comptus* it is evidently felted, sometimes with hyphae aggregated in tufts under the observation with magnifying glass (Fig. 5d); under light microscopy, hyphae terminal elements appear often loose and prostrate, running more or less parallel to the pileus surface (Fig. 5e), whereas in *B. mendax* the trichodermium presents hyphae substantially erect and parallel, persistently arranged in this way until maturation of the basidiomata (Fig. 5g, h).

The autonomy of the taxon *B. comptus* as an independent species is here demonstrated by the well independent clade consisting of two collections coming from different areas (see “Molecular results” in chapter “Results” and Fig. 1).

“*Boletus caucasicus*” is still a nomen nudum. All the following validation attempts have failed: *Boletus* ‘*erythropus-luridus*’ Singer (1931) nom. inval. (Art. 23.6, Melbourne code, McNeill et al. 2012, <http://www.iapt-taxon.org/nomen/main.php>); *Boletus luridus* ‘var. *caucasicus*’ Singer (1947) nom. inval. (Art. 39.1); *Boletus* ‘*caucasicus*’ Singer (1967) nom. inval. (Art. 39.1); *Boletus* ‘*caucasicus*’ Singer ex Alessio (1985) nom. inval. (Art. 40.1, 40.3); *Boletus luridus* ‘subsp. *caucasicus*’ (Singer ex Alessio) Hlaváček (1995) nom. inval. (Art. 41.5).

Nevertheless, it is challenging to understand the correct original concept for this name. From Singer’s 1931 description, the most characterizing features are (translated from German): “In mixed woods, under *Abies normanniana*. Pileus pale gray-brown, darker than in *Boletus luridus*. Pores bright Venetian-red, darker than in *B. luridus* and paler than in *B. erythropus*. Stipe reddish-pink in the upper part, with a reticulum. In the lower part with small red granules, progressively reducing downwards, olive to dirty blood red at the base. Context lemon yellow with areas with a more intense colour. Layer below the tubes of the same colour of the context. Taste and smell not distinctive”. Some features of this description might agree with *B. mendax*, in particular the stipe colour, the red punctation of the lower part of the stipe, the colour of pores and subhymenophoral layer (defined as yellow in the original diagnosis), which is a feature found occasionally in *B. mendax*. Singer’s (1947) paper, with the proposal of a nomen novum as a variety of *B. luridus*, based on *Boletus erythropus-luridus*, introduces for the first time the epithet “*caucasicus*”; Singer’s (1967) description is enriched with two significant characters: the negative amyloid reaction and the indication of the spores length/width ratio higher than that of *B. luridus*, both typical character of *B. luridiformis* (= *B. erythropus* s. auct.), however only the spores shape results compatible with *B. mendax*, which conversely displays a strongly positive amyloid reaction, but there is no evidence that these characters were already present in the 1931 collections. These same characters were mentioned for the “*Bolét de Quélet* a pied reticulé” by Imler (1934), a taxon identified as *B. caucasicus* by Singer (1947).

Even if from Singer’s 1931 and 1967 descriptions a well defined and characterized taxon seems to emerge due to a series of relevant characters (negative amyloid reaction, spores with a high length/width ratio), collections putatively ascribable to *B. caucasicus* appear indeed to fall, except for the yellow sub-hymenophoral layer, within the range of *B. luridus* owing to the positive amyloid reaction of tissues and the low spore length/width ratio (ranging within 2.1–2.4). These collections, infrequent but not rare, are often called “*B. caucasicus* Singer” (i.e. Cetto 1976; Cetto 1982) and one of them (MCVE17696) resulted fully included in *B. luridus* clade in the phylogenetic analysis (Fig. 1). This evidence undoubtedly demonstrates that, even in *B. luridus*, the occasional lack of the Bataille’s line is not a significant taxonomic character.

By contrast, we believe that *B. caucasicus* in the original Singer’s concept might represent a *B. luridiformis* phenotype having a more or less outlined reticulum, all remaining features (such as

negative context amyloid reaction and spores length/width ratio ranging within 2.5–2.9) being typical and diagnostic for the latter species (Simonini 2001).

Boletus queletii

Schulzer (Schulzer 1885) is a well-defined and non-problematic species differing from *B. mendax* in having no Bataille's line, no reticulum, pores with brick-orange tints, spores ellipso-amygdaliform with average dimension $12 \times 5.7 \mu\text{m}$, $Q = (1.8\text{--})1.9\text{--}2.3\text{--}(2.4)$ (Muñoz 2005) ($Q = 2.0\text{--}2.2$; $Q_m = 2.1$; $c = 12$, pers. obs.), the less elongated in this group, and a pileipellis consisting of a confused trichodermium (Muñoz 2005 and pers. obs.). *B. queletii* var. *pseudoluridus* J. Blum (Blum 1968) shows a stipe dotted with fine meshes, without reticulum, but with the context clearly orange under the tubes. In our opinion these collections have to be included in *B. luridus* variability range rather than within *B. queletii*. Muñoz interpretation of this taxon (2005), presenting a stipe with dots forming a reticulum and no pigmentation under the tubes, is unknown to us.

Some boletes included in sect. *Luridi* formerly described for the European mycological flora, such as *Boletus rubeolarius* Bull., *B. tuberosus* Bull. (Bulliard 1791), *Tubiporus lividorubricosus* Paulet (1793), *B. purpureus* Secretan, *B. fuliginosus* Secretan (Secretan 1833), *B. sordarius* Fr. (Fries 1838), *B. tessellatus* Rostk., *B. dictyopus* Rostk., *B. meyeri* Rostk., *B. luridiformis* Rostk. (Rostkovius 1844), *B. lateritius* Bres. & Schulzer, *B. queletii* var. *squarrosipes* Schulzer, *B. bresadolae* Schulzer (Schulzer 1885), *Boletus aetnensis* Inzenga (1869), *B. rutilus* Fr. var. *queletii* Schulzer (1885), *B. jandae* Schulzer (1887), *B. queletii* Schulzer var. *rubicundus* Maire (1910), *B. melzeri* Velen. (Velenovský 1922), *B. smotlachianus* Hlaváček (1983), share some character with *B. mendax*, but none of them provides a set of features capable to make it a realistic previous concurrent of our new species.

Among extra-european boletes belonging to the sect. *Luridi*, the closest relatives to *B. mendax* seem to be *B. subvelutipes* Peck, *B. craspedius* Masee and *B. sinicus* W.F. Chiu.

B. subvelutipes (Peck 1889), well illustrated in Bessette et al. (2000), however has larger size, larger spores, brighter pores and stipe colours as well as a stipe very rarely with a reticulum (Singer 1947; Smith and Thiers 1971): *B. craspedius* (Masee 1914), well illustrated in Corner (1972), differs by its larger size, smaller spores, pileus margin becoming paler in age and pileipellis cells with epiparietal encrustation. *Boletus sinicus* (Chiu 1948, 1957; Wang et al. 2004) shows an overall appearance that might suggest a relationship with *B. mendax* (medium size, garnet-red stipe and pileus, reticulum over the stipe). However the spore size is quite different, $7.5\text{--}11 \times 4.5\text{--}5.5 \mu\text{m}$ (in average $9.5 \times 4.5 \mu\text{m}$), the pileus is said "fibrillose scaly" as it is covered by gray membranose-like scales; the context is white, yellowish in the stipe, turning blue when cut but with no red tones at the stipe base and it is usually found under *Pinus yunnanensis* (Gang Wu, Kunming Institute of Botany, China, Pers. comm.).

Among the other morphologically allied boletes, *B. miniato-olivaceus* Frost (1874), *B. miniato-olivaceus* var. *subluridus* (Murrill) Singer, *B. austrinus* Singer, *B. hypocarycinus* Singer (Singer 1945), *B. magnificus* W.F. Chiu (1948), *B. reayi* Heim (1963), *B. loosii* Heinemann (1964), *B. quercinus* Hongo (1967), *B. floridanus* (Singer) Murrill (Murrill 1948; Both 1993; Bessette et al. 2000; Ortiz-Santana et al. 2007) and *B. kermesinus* Har. Takah., Taneyama & Koyama (Takahashi et al. 2011), none of them could represent a possible concurrent of *B. mendax*.

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